



**QUEEN'S
UNIVERSITY
BELFAST**

Mycotoxin co-exposures in infants and young children consuming household- and industrially-processed complementary foods in Nigeria and risk management advice

Ojuri, O. T., Ezekiel, C. N., Eskola, M. K., Šarkanj, B., Babalola, A. D., Sulyok, M., Hajšlová, J., Elliott, C. T., & Krska, R. (2019). Mycotoxin co-exposures in infants and young children consuming household- and industrially-processed complementary foods in Nigeria and risk management advice. *Food Control*, 98, 312-322.
<https://doi.org/10.1016/j.foodcont.2018.11.049>

Published in:
Food Control

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights

Copyright 2018 the authors.
This is an open access article published under a Creative Commons Attribution-NonCommercial-NoDerivs License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.



Mycotoxin co-exposures in infants and young children consuming household- and industrially-processed complementary foods in Nigeria and risk management advice

Oluwaseun T. Ojuri^a, Chibundu N. Ezekiel^{a,b,*}, Mari K. Eskola^b, Bojan Šarkanj^{b,c}, Akinola D. Babalola^d, Michael Sulyok^b, Jana Hajšlová^e, Christopher T. Elliott^f, Rudolf Krska^{b,f}

^a Department of Microbiology, Babcock University, Ilishan Remo, Ogun State, Nigeria

^b Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad Lorenzstr. 20, A-3430, Tulln, Austria

^c Department of Food Technology, University North, Center Koprivnica, Trg dr. Zarka Dolinara 1, HR – 48000, Koprivnica, Croatia

^d Department of Agriculture and Industrial Technology, Babcock University, Ilishan Remo, Ogun State, Nigeria

^e University of Chemistry and Technology, Prague, Czech Republic

^f Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, University Road, Belfast, BT7 1NN, Northern Ireland, United Kingdom

ARTICLE INFO

Keywords:

Complementary foods
Consumer awareness
Exposure and risk management
Food safety
Infant nutrition
Mycotoxins

ABSTRACT

This study compared mycotoxin levels in 53 household-formulated and 84 industrially-processed complementary foods, assessed co-exposure patterns from consumption of the contaminated foods by infants and young children (IYC) in two Nigerian states, and evaluated the influence of awareness and adopted processing practices at the household levels on toxin levels in the foods. About 42 and 93% of the industrial- and household-processed foods, respectively, were contaminated by mycotoxins. Aflatoxins, alternariol, citrinin and dihydrocitrinone levels were significantly higher in household-formulated foods while fumonisins were similarly higher in the industrially-processed foods. Of the household-formulated items, *Tom bran* contained higher aflatoxin levels leading to higher exposure (median: 641 ng/kg bw per day) and health risk (β -coefficient: 51.4; $p = 0.01$) in the IYC. Family cereal and *ogi* contained the highest levels of fumonisins in the industrial and household food categories, respectively, with the highest exposure estimated for IYC who consumed family cereal (median: 18 μ g/kg bw per day). Aflatoxin exposures were higher in children aged 12–24 months compared to those below 12 months of age. About 69 and 75% of IYC who consumed family cereal and *Tom bran*, respectively, were co-exposed to mycotoxins resulting in commensurate risks of co-exposures. Overall, 47% of the IYC were co-exposed to 2–4 mycotoxins (aflatoxins, citrinin, fumonisins and ochratoxin A) with eight different co-exposure combinations. Only 33% of the respondents were aware of mycotoxins. Length of grain storage influenced food aflatoxin levels. Adequate risk management advice to concerned stakeholders for mycotoxin control in complementary foods in Nigeria is offered herein.

1. Introduction

Mycotoxin contamination of food continues to pose a major challenge to food safety, especially in economically developing regions such as sub-Saharan Africa (SSA) (IARC, 2015). This is due to a complex set of factors summarized as poor agricultural inputs at the pre-harvest stage, poor food handling and processing, poverty and heavy reliance of home-grown cereals as food for both adults and children, low level of awareness of the mycotoxin problem, minimal incentives to drive education and awareness of mycotoxins, and lack of adequate

mycotoxin regulations (IARC, 2015). It is known that a single crop (e.g. maize) can be prone to several mixtures of mycotoxins such as aflatoxins, citrinin, fumonisins, ochratoxins and the trichothecenes (Adetunji et al., 2014; Njumbe-Ediage, Hell, & De Saeger, 2014; Okeke et al., 2015; Oyedele et al., 2017; Warth et al., 2012), and most times, foods consumed at the household level irrespective of the source (home-made or industrially-processed) are a combination of diverse mycotoxin prone crops. Consequently, large proportion of individuals living in high risk regions such as SSA become heavily exposed to a cocktail of mycotoxins via their diets, children inclusive.

* Corresponding author. Department of Microbiology, Babcock University, Ilishan Remo, Ogun State, Nigeria.

E-mail address: chauguez@gmail.com (C.N. Ezekiel).

<https://doi.org/10.1016/j.foodcont.2018.11.049>

Received 16 September 2018; Received in revised form 7 November 2018; Accepted 26 November 2018

Available online 27 November 2018

0956-7135/ © 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The diets of infants and young children (IYC; children within 24 months of age) range from breast milk to cereal- and nut-based complementary foods (Gong, Watson, & Routledge, 2016; IARC, 2015). The cereal- and nut-based complementary foods consumed in many SSA countries, including Nigeria, are made mostly from low priced grains (Kamala et al., 2017; Kimanya et al., 2010, 2009; Kimanya et al., 2014; Matumba et al., 2014), and the cereal and nut content requirements of the foods increase as ages of the IYC increase; thus placing this group at risk of the adverse effects of mycotoxins. Ideally, complementary foods are recommended diets for IYC from six months of age; however, in many low- and middle-income countries, complementary foods are usually introduced earlier than recommended which tend to increase childhood exposure to mycotoxins (Gong et al., 2016; IARC, 2015). In Nigeria, a variety of complementary foods are available for consumption by IYC and they include cereal-based foods, milk, infant formula and nut-based foods. All the aforementioned food types can be processed by industries while the cereal- and nut-based foods are formulated singly or in combination at household level. Depending on the income of the families, preference for complementary foods varies; this may include industrially-processed, household-formulated, or a combination of both categories.

Childhood exposure to mycotoxins have been associated with poor child growth and development, and increased susceptibility to infections amongst many other adverse effects (Gong et al., 2002; Gong et al., 2004; IARC, 2015; Kamala et al., 2017; Kimanya et al., 2010, 2009; Kimanya et al., 2014; Shirima et al., 2015; Turner, 2013; Turner, Moore, Hall, Prentice, & Wild, 2003; Turner et al., 2007). There are also evidences of the potential adverse health risks that may face individuals who are co-exposed to mycotoxin mixtures; these range from synergistic toxicity damages to complex additive effects in diverse body cells and organs (Clarke, Connolly, Frizzell, & Elliott, 2014; Clarke, Connolly, Frizzell, & Elliott, 2015; Creppy et al., 2004; Golli-Bennour et al., 2010; Klarić, Rumora, Ljubanović, & Pepelnjak, 2008; Klarić et al., 2012; Stoev, Denev, Dutton, & Nkosi, 2009). IYC are thus more vulnerable to these adverse effects due to a poorly developed immune system, high rate of metabolism, and fairly restricted diet (Gong et al., 2016; Martani, 2014).

Mycotoxin contamination of complementary foods for IYC have been reported in many countries including our recent report on these foods in Nigeria (Alvito, Sizoo, Almeida, & Egmond, 2010; Baydar, Erkekoglu, Sipahi, & Sahin, 2007; Juan, Raiola, Mānes, & Ritieni, 2014; Kabak, 2009; Kamala et al., 2017; Kimanya et al., 2010; Kimanya et al., 2014; Ojuri et al., 2018; Okoth & Ohingo, 2004; Tam et al., 2006). However, there is paucity of information on comparison of multiple mycotoxin contamination of complementary foods formulated at household level to those processed by industries in Nigeria. In addition, minimal information exists on the extent and patterns of co-exposures and risks of co-exposures to mycotoxins from these food sets, as well as lack of data for awareness assessments of caregivers to IYC on mycotoxin issues and the influence of food processing practices on mycotoxin content of household-formulated food sets. Consequently, this study aimed to: a) assess mycotoxin co-exposures and risks of co-exposures from consumption of household-formulated and industrially-processed complementary foods in IYC living in Lagos and Ogun states, Nigeria, and b) evaluate awareness levels of caregivers to the IYC and the role of food processing practices adopted in the household setting on mycotoxin levels in the foods. This study further offers risk management advice to all stakeholders for effective mycotoxin control in the complementary food chain in Nigeria.

2. Materials and methods

2.1. Survey

A socio-demographic survey was conducted among voluntary households in Ikorodu (Lagos state) and Ilishan Remo (Ogun state),

Nigeria in order to collect complementary food samples fed to IYC in these states, assess the caregivers' awareness of mycotoxins, and understand the IYC feeding patterns and food storage practices adopted by the caregivers. The two states were selected for this study based on their proximity to each other and easy accessibility to conduct this study. A total of 110 households with IYC of 6–24 months old were randomly selected for this study, although five infants (< 6 months of age) were included in the study due to early introduction of complementary foods in their diets. Household participation in the study was voluntary. Each household was informed of the objectives and scope of the study prior to their inclusion. Only IYC without known ill-health as indicated by their caregivers were included in the study, after appropriate documented consent was given by the caregivers. The study was approved by the Babcock University Health Research Ethics Committee (BUHREC) under the authorization number 524/17.

During the survey, a well-structured food frequency and mycotoxin awareness questionnaire was administered to each household to obtain data on socio-economic status of households, anthropometric data, food consumption pattern, dietary preference, health status of the IYC in the 110 households selected for the study, and the mycotoxin awareness of caregivers of the IYC. The caregiver of each child completed the questionnaire and provided data on their children for the purpose of the study.

2.2. Mycotoxin analysis of complementary foods

A total of 137 complementary food samples (84 industrially processed and 53 household-formulated foods) were collected from the participating households in January and July 2017 and analyzed for mycotoxin contamination levels. The distribution of the food types into their categories based on processing include: industrially processed (family cereal ($n = 26$), peanut butter ($n = 5$), powdered milk ($n = 36$) and infant formula ($n = 17$)) and household-formulated (*ogi* ($n = 23$) and *Tom bran* ($n = 30$)). Family cereal is a maize product while infant formula included products with a mix of milk and cereal (e.g. maize, oats, rice or wheat) depending on the brand. *Ogi* is a maize-based fermented gruel while *Tom bran* is usually formulated from several whole grains including maize, peanuts, wheat, soybean and millets. Family cereal, infant formula, *ogi* and *Tom bran* are consumed as pudding while milk and peanut butter are minimally consumed due to their use as supplements. Other details of food samples from the households are as described in Ojuri et al. (2018).

For mycotoxin analysis, briefly, 5g of the 20g food samples collected were homogenized, extracted with 20 ml of acetonitrile/water/acetic acid (79:20:1, v/v/v) and injected directly into the LC-MS/MS instrument according to the “dilute and shoot” method described by Sulyok, Krska, and Schuhmacher (2007). Other details related to LC-MS/MS screening and parameters are as described by Malachová, Sulyok, Beltrán, Berthiller, and Krska (2014), while spiking, recovery and accuracy of the method were previously reported in Ojuri et al. (2018).

2.3. Food item-driven mycotoxin exposure and risk assessment of IYC

2.3.1. Exposure assessment

The objective of the exposure assessment in this study was to evaluate the contribution of individual food items to exposure and co-exposure of IYC to mycotoxins. The deterministic approach using the probable daily intake (PDI) method for assessing exposure of chemicals occurring in foods (Codex Alimentarius, 1989; IPCS, 2009) was adopted in this study to assess the chronic exposure of IYC to various mycotoxins (single and co-occurring) in the complementary food items they frequently consumed. Data on daily consumption of complementary foods (g/day) were obtained for the 110 IYC recruited into the study and the food consumption of each child was based on the complementary food item that was most frequently consumed by the child as described in Ojuri et al. (2018). Similarly to Ojuri et al. (2018) the actual mycotoxin

concentration determined for the specific food item was used, with the exception that for food item sample reporting concentrations < LOD either LOD/2 (middle bound) or 0 ng/kg was applied. This was considered to give an appropriate exposure estimate (IPCS, 2009) and to simplify the overall assessment of risks from the (co-)exposures. The above approach was used for exposure estimation of each infant to the individual mycotoxins in each food item consumed. In order to determine co-exposures to mycotoxins resulting from the consumption of different food items by each child, the different mycotoxins to which each subject was simultaneously exposed were counted.

2.3.2. Risk characterization

The risk characterization and overall risk assessment was conducted according to the internationally accepted protocols, including uncertainty evaluation, and was considered sufficiently robust as concluded previously in Ojuri et al. (2018). The minor difference between the present study and the previous study was the use of a middle bound for all left-censored data (data below < LOD) instead of the lower-bound/upper-bound approach for ochratoxin A. This, however, does not change this previous conclusion, and the impact of the use of the middle bound on uncertainty of the risk assessment remains the same as in Ojuri et al. (2018). Namely, the application of the middle bound can either underestimate or overestimate the exposure as the left-censored samples may have contained mycotoxins at higher levels than the middle bound or they could have been free from mycotoxins. Thus, depending on the exerted toxicity of the mycotoxin and the reliability of the available toxicity data, the risk characterization was performed either by applying a margin of exposure (MOE) approach or by comparing the exposure to the established health based guidance value (HBGV) as presented in Ojuri et al. (2018). To categorize whether the exposures to mycotoxins posed health risks or not for the IYC population, the established HBGVs were used as a divider (i.e. when exposure is above HBGV, risk occurs and when below, no risk) in the risk assessment. The MOE approach was adopted for the mycotoxins with uncertainties in the toxicological database ((BEA, MON and CIT) (see Ojuri et al., 2018)). For categorizing the risk from the exposure to these mycotoxins, it was considered that the risk did not occur when the MOE was above 100 but when MOE was below 100, risk occurred. This approach was considered appropriate as the selected reference points for MOE calculations were deemed conservative. However, for CIT a concern for genotoxicity and carcinogenicity remains at the exposure level of the applied reference point as concluded by EFSA (2012). Aflatoxin B₁ is the only mycotoxin which has been confirmed to be a genotoxic-carcinogen to humans (EFSA, 2007; IARC, 2015). For substances which are both genotoxic and carcinogenic, and therefore can pose health risks at any dose level, MOE of 10,000 was applied (Benford et al., 2010; EFSA, 2005, 2007). To categorize the health risk from the dietary exposure to aflatoxin B₁ or from the sum of aflatoxins, the MOE of 10,000 or larger was regarded as low risk and below 10,000 as risk. This was considered suitable based on EFSA (2005), which recommended that a MOE below 10,000 for a genotoxic-carcinogen (based on calculated benchmark dose limit from an animal study) is regarded as an indication that the exposure to this genotoxic-carcinogen is of a potential public health concern and requires risk management actions.

2.4. Assessment of mycotoxin awareness and food processing practices among infant caregivers

In order to assess the mycotoxin awareness level of caregivers to the infants and young children fed with complementary foods, and establish the relationship between adopted food processing practices at household levels and mycotoxin levels in the household-formulated complementary foods, regression analyses were performed on data obtained from questionnaire administration during the survey. This was necessary to identify the factors influencing the levels of mycotoxins in

the complementary food samples. Consumers' awareness of mycotoxin contamination of food was regressed on consumer specific characteristics (e.g. educational level of respondents, respondents' perceived risk of mycotoxin contamination from previous personal experience, and use of food product label (i.e. description and instructions for use of food)) in order to identify the factors determining awareness. This was achieved using the logit regression model following Babalola, Babalola, and Bassey (2010) and Gujarati (2003). The model is specified as follows:

$$\ln(P_i/(1-P_i)) = \beta_0 + \beta_1 X_1 + \dots + \beta_n X_n + e_i$$

Where P_i = Probability of mothers' awareness of mycotoxin contamination in food, β_1 = coefficients, X_i ($X_1 \dots X_n$) = independent variables and e_i = error term. The independent variables which describe the use of complementary food product label, experience with contaminated food and respondents' education are described as follows:

$$Y = f(X_1, X_2, X_3 \dots U)$$

Where.

Y = Mothers' awareness of mycotoxin contamination of food (Dummy: aware = 1, otherwise = 0)

X_1 = Respondents' education (years)

X_2 = Use of complementary food product label (Dummy: Yes = 1, No = 0)

X_3 = Perceived risk from personal experience with contaminated food (Dummy: Yes = 1, No = 0).

2.5. Statistical analysis

All the data were analyzed using SPSS Statistics package version 20.0 (SPSS Inc., Chicago, IL, USA). Food consumption data were analyzed using descriptive statistics while the analysis of variance (ANOVA) and the unpaired student t-test (two-sided) were used to compare mean mycotoxin levels in the complementary foods grouped based on their processing types (industrially processed and household-formulated). The Duncan's Multiple Range test (DMRT) at 95% confidence level was applied as post hoc test to separate significant values ($p < 0.05$). Statistica version 13.3 (TIBCO Software, Palo Alto, CA, USA) was used for comparisons between groups and box plots, while Flourish studio was used for Sankey diagrams (Kiln Enterprises Ltd, London, UK).

3. Results and discussions

3.1. Demographic and complementary feeding practice data for the infants and young children

The demographic data obtained from the 110 respondents (caregivers on behalf of their IYC) as well as the feeding practices adopted for the IYC are highlighted in Table 1. The age of the children ranged 6–24 months; 49% of the children were below 12 months of age while 51% were aged 12–24 months. The mean (\pm SD) body mass index (kg/m²) of the children by age groups were 16.9 ± 2.9 and 18.2 ± 3.6 for children below 12 months and 12–24 months of age, respectively. The children consumed the sampled complementary foods 1–6 times daily. Based on the category of complementary food consumed, 60% of the children were fed both household-formulated and industrially-processed complementary foods, while approximately one-fifth each of all 110 children were fed with either of the food categories. The frequently consumed food items were *Tom bran*, family cereal and *ogi*, and 27, 24 and 21% of the children ate these food items, respectively, on the day of sampling. Higher proportions, 25 and 32%, of the children in the age range of 12–24 months consumed family cereal and *Tom bran*, respectively, than other food items while majority (26%) of the children

Table 1
Basic descriptive statistics of respondents' characteristics and complementary feeding practices in Nigeria.

Variables	Frequency (n = 110)	%
<i>Sex of children (dummy)</i>		
1 = Male	60	54.5
2 = Female	50	45.5
Age (mean ^a months) of children	14.6 ± 6.3	
Body weight (mean ^a kg) of children	9.8 ± 2.2	
Height (mean ^a cm) of children	74.9 ± 8.7	
Body Mass Index (kg/m ²) of children (range; mean ^a)	11.2–27.3	17.6 ± 3.4
< 12 months of age	11.2–23.8	16.9 ± 2.9
12–24 months of age	13.2–27.3	18.2 ± 3.6
Children with health challenge	0	0
Age of complementary food introduction (mean ^a weeks)	18.3 ± 1.6	
<i>Category of complementary food consumed</i>		
Household-formulated	24	21.8
Industrially processed	21	19.1
Both	65	59.1
<i>Children consuming complementary food items</i>		
Tom bran	30	27
Family cereal	26	24
Ogi	23	21
Infant formula	15	14
Milk	11	10
Peanut butter	5	4
<i>Consumption frequency</i>		
1–3 times per day	60	54.5
4–6 times per day	50	45.5
<i>Complementary food intake (g) (range; mean^a)</i>		
< 12 months of age	150–870; 550 ± 184	
12–24 months of age	250–950; 666 ± 156	
Weight of food consumed (mean ^a kg)	0.61 ± 0.18	
<i>Education of Mother (years)</i>		
Mean ^a	14.5 ± 3.4	
Informal	0	1.8
Formal	16	98.2

^a Mean ± Standard deviation.

below 12 months of age consumed *ogi*. The mean (± SD) daily intake of complementary foods was slightly higher in the children aged 12–24 months (666 g ± 156) than in those below 12 months of age (550 g ± 184). This was mainly due to the higher food consumption of older IYC, owing to their higher energy requirements, and the consumption of *Tom bran* which is a heavy weighted but rapidly metabolized meal even at an expected “reasonable” meal portion for IYC. In most families, *Tom bran* is usually introduced to IYC at the age of 6 months. As reported in our previous paper, Ojuri et al. (2018), IYC in Ilishan Remo and Ikorodu are introduced to complementary foods as early as the third month from birth; this depends mainly on the family income and capacity for exclusive breastfeeding practice. It has previously been reported that socio-economic factors such as background/education and family income may play a role in early introduction of complementary foods to IYC (Lindsay, Machado, Sussner, Hardwick, & Peterson, 2008). Approximately 98% of the mothers to the IYC had formal education with mean (± SD) educational years of 14.5 ± 3.4, indicating at least high school/secondary level of education. The levels of education recorded for the mothers in this study are obviously similar to our previous reports for education levels of peanut cake consumers in Lagos and Ogun states (Ezekiel et al., 2013).

3.2. Major mycotoxins in household-formulated and industrially-processed complementary foods

The variations in occurrence levels of 23 mycotoxins (i.e., 21 individual mycotoxins in addition to the sum of aflatoxins (B₁, B₂, G₁ and G₂) and sum of fumonisins (B₁, B₂ and B₃)) found in the complementary

food samples are presented in Table 2. In this paper, we only present mycotoxin concentrations based on category of processed food (household-formulated and industrially-processed foods); detailed occurrence of mycotoxins and other multiple microbial metabolites in the sampled food items are given in our recent paper (Ojuri et al., 2018). As much as 93% of the household-formulated complementary food samples contained mycotoxins while only up to 42% of the industrially-processed foods were found to be contaminated. The mean concentrations of aflatoxins (AFB₁, AFB₂, AFG₁ and sum of aflatoxins), alternariol, citrinin and dihydrocitrinone were significantly ($p < 0.05$) higher in household-formulated complementary foods than in the industrially-processed, while the mean levels of the fumonisins were significantly ($p < 0.05$) higher in the industrially-processed foods than in the household-formulated foods.

The occurrence and higher levels of several mycotoxins in household-formulated complementary foods compared to the contamination levels in the foods from the industry, excluding the case of fumonisins, point to the dangerous roles of poor grain storage conditions and exclusion of simple mycotoxin reduction strategies (e.g. drying to safe moisture content and sorting out discolored or insect infested grains), which are common practices in the handling of foods at household levels in SSA (Adetunji et al., 2014; Kang'ethe et al., 2017; Okeke et al., 2015), in the safety of food. The roles of poverty and food insufficiency, which drive the use of obviously damaged/low quality grains as raw materials for food at the household level, should not be overlooked. It is known that the industries often source high quality grains, have good storage conditions that are routinely monitored, and apply stringent quality control checks targeted at preventing mycotoxin contamination. However, the lack of regulation for other toxins, other than aflatoxins, in diverse food items in SSA may have accounted for the increased levels of fumonisins in the industrially-processed complementary foods. Additionally, protective measures (e.g. routine monitoring of grains) taken by the industries were obviously focused on aflatoxins, thus excluding measures against field-formed fumonisins. With respect to food items in each food category (household and industrial; data not shown), *Tom bran* and *ogi* contributed the most to aflatoxin levels in household foods while family cereal and peanut butter had the higher shares of aflatoxin levels in the industrial products. For fumonisins, *ogi* and family cereal were the two food types with higher levels in the household and industrial products categories, respectively.

3.3. Estimated mycotoxin exposures and risks due to contaminated complementary foods

3.3.1. Food item-dependent exposures, co-exposures and associated risks in IYC

The mycotoxin exposures, based on LOD/2 replacements for mycotoxin contamination data points that were less than LOD per food item fed to the 110 children, are presented in Table 3. Exposure to aflatoxins (sum of aflatoxins) was highest in children who consumed *Tom bran* (median: 641 ng/kg bw per day), although children who consumed other food items were also exposed to aflatoxins as depicted in the trend: *Tom bran* > peanut butter (median: 441 ng/kg bw per day) > family cereal (median: 179 ng/kg bw per day) > *ogi* cereal (median: 68 ng/kg bw per day) > infant formula (median: 50 ng/kg bw per day). Daily exposure to aflatoxins for a significant period of time may lead to the development of hepatocellular carcinoma, stunting and other chronic health conditions (IARC, 2015). This is of a particular concern considering the young age of the consumer group in this study. It should be noted that the contamination and exposure levels from *ogi* and *Tom bran* may be reduced by a factor of 0.5 in view of a 1:1 (w/v) dilution with water that occurs during the preparation (i.e. prior to heat-treatment) of these two foods. However, exposure of the IYC via these foods should not be downplayed since aflatoxin concentrations were generally high in the samples and because of their regular consumption by this vulnerable population.

Table 2

Variations in mycotoxin levels in household-formulated and industrially-processed complementary foods in Nigeria.

Mycotoxins	Household product (n ^a = 53)				Industrial product (n ^a = 84)			
	%p ^b	Concentration (µg/kg)			%p ^b	Concentration (µg/kg)		
		Range	Median	Mean ± SD ^c		Range	Median	Mean ± SD ^c
Aflatoxicol	11.3	1.4–7.8	3.7	4.4 ± 2.7	0.0	< LOD	–	–
Aflatoxin B ₁	67.9	0.4–474	5.7	57.2 ± 10.8 a	34.5	0.4–11.6	2.0	3.2 ± 3 b
Aflatoxin B ₂	34.0	0.6–81.8	4.2	12.1 ± 19.2 a	11.9	0.5–2	0.7	0.9 ± 0.5 b
Aflatoxin G ₁	45.3	0.4–237	1.3	15 ± 48.3 a	28.6	0.4–2.5	0.9	1 ± 0.5 b
Aflatoxin G ₂	7.5	1.4–20.7	3.4	7.2 ± 9.1	0.0	< LOD	–	–
Sum of aflatoxins ^d	69.8	0.4–590	7.0	72 ± 14.4 a	36.9	0.4–13.6	2.6	4.1 ± 3.5 b
Aflatoxin M ₁	28.3	0.9–24.4	3.0	5.1 ± 6.2	0.0	< LOD	–	–
Alternariol	18.9	0.4–7.2	1.3	1.9 ± 1.9 a	6.0	0.4–0.9	0.6	0.6 ± 0.2 b
Beauvericin	90.6	0.1–69	0.7	3.2 ± 10.3 a	41.7	0.04–13.4	0.2	0.8 ± 2.3 a
Citrinin	67.9	0.8–1173	9.5	106 ± 25.4 a	28.6	1.2–151	21.9	31.4 ± 39.4 b
Dihydrocitrinone	32.1	2.4–210	12.0	30.3 ± 49.9 a	2.4	2.2–3.4	2.8	2.8 ± 0.8 b
Deoxynivalenol	3.8	30.8–31.6	31.2	31.2 ± 0.6 a	2.4	27.2–36	31.6	31.6 ± 6.3 a
Fumonisin A ₁	28.3	1.2–11.3	3.2	3.9 ± 3	0.0	< LOD	–	–
Fumonisin A ₂	34.0	3.2–42.3	13.1	15 ± 10.7 a	20.2	2.3–42.6	16.2	16.7 ± 10.3 a
Fumonisin B ₁	86.8	11–974	84.5	152 ± 186 a	31.0	43–836	176.4	245 ± 195 a
Fumonisin B ₂	86.8	7.1–403	34.8	76.6 ± 88 b	31.0	19.3–267	60.4	84.5 ± 62.7 a
Fumonisin B ₃	54.7	7.4–143	30.6	40.1 ± 30.2 b	28.6	12.4–152	48.2	48.3 ± 35.9 a
Sum of fumonisins ^e	92.5	7.8–1436	114.5	238 ± 292 b	31.0	62.3–1255	265.4	374 ± 294 a
Fumonisin B ₄	67.9	3.7–222	29.4	39 ± 42 a	29.8	7.3–109	28.0	33.8 ± 25.7 a
Moniliformin	62.3	2.4–3450	27.5	146 ± 596 a	34.5	1.7–34.8	7.8	9.3 ± 7.2 a
Nivalenol	5.7	11.4–23.8	14.1	16.4 ± 6.6 a	2.4	18.9–22	20.5	20.5 ± 2.2 a
Ochratoxin A	18.9	0.5–26.4	2.6	5.7 ± 8.2 a	2.4	0.5–0.5	0.5	0.5 ± 0 a
Zearalenone	11.3	0.4–10.3	3.2	3.7 ± 3.6 a	6.0	0.4–5.4	3.0	2.7 ± 2.2 a

^a Number of samples analyzed.^b Percent positive samples.^c Mean and standard deviation from mean of toxin levels found in the foods.^d Summation of aflatoxins B₁, B₂, G₁ and G₂.^e Summation of fumonisins B₁, B₂ and B₃. Mean values in a row with different alphabets are significantly different at $\alpha = 0.05$.

Consumption of family cereal, *Tom bran* and *ogi* also resulted in higher median fumonisin exposures of 18 µg/kg bw per day, 8.2 µg/kg bw per day and 6 µg/kg bw per day, respectively, than consumption of infant formula 0.3 µg/kg bw per day (range: 0.13–0.47 µg/kg bw per day) which was below the group TDI of 2 µg/kg bw per day for the sum of FB₁, FB₂, FB₃ and FB₄ (Table 3). The principal grain component in each of the three food items that resulted in high fumonisin exposure in the IYC is maize. Maize and maize products (including complementary foods) from countries within SSA have been reported to be heavily contaminated with fumonisins leading to high exposures in the African

population, especially among children (Adetunji et al., 2014; Kamala et al., 2017; Kimanya, De Meulenaer, Tiisekwa, Ndomondo-Sigonda, & Kolsteren, 2008; Kimanya et al., 2010; Kimanya et al., 2014; Mngqawa et al., 2016; Okeke et al., 2015). The exposures reported for these food items are quite high considering that the food items are already processed for consumption and the group TDI for this toxin is exceeded by several folds. Consequently, it is paramount to consider priority actions towards mitigation and legislation of this mycotoxin whose regulation is almost non-existent in many foods, especially those intended for IYC, in several of the SSA countries including Nigeria.

Table 3

Complementary food item-dependent mycotoxin exposures in 110 infants and young children in Nigeria.

Mycotoxins	Exposure levels ^a	Complementary food items				
		<i>Tom bran</i>	Peanut butter	<i>Ogi</i>	Infant formula	Family cereal
Aflatoxin B ₁	Range	5.5–51,192	6.6–1079	5.7–3211	3.5–426	2.5–639
	Median	528	349	20	7	91
Sum of aflatoxins ^b	Range	40.5–54,892	48.4–1317	41.8–3539	25.7–533	27–902
	Median	641	441	68	50	179
Sum of fumonisins ^c	Range	0.27–138.6	–	0.31–55.8	0.13–0.47	2.9–98.5
	Median	6	–	8.2	0.3	18
Ochratoxin A	Range	0.0–2.03	0.02–0.04	0.0–0.1	0.01–0.04	0.0–0.05
	Median	0.02	0.02	0.03	0.02	0.02
Citrinin	Range	0.0–102	0.0–0.01	0.0–8.1	0.0–0.5	0.0–13.6
	Median	0.4	0	0.1	0	0.6
Moniliformin	Range	0.04–156.8	0.04–0.28	0.01–1.5	0.02–0.8	0.03–1.63
	Median	2	0.15	0.1	0.05	0.5
Beauvericin	Range	0.0–3.14	0.0–0.33	0.0–0.4	0.0–1.4	0.0–0.03
	Median	0.04	0.04	0.02	0	0.01

^a Exposures in ng/kg bw per day for aflatoxins; µg/kg bw per week for ochratoxin A; µg/kg bw per day for other mycotoxins. To derive exposures, mycotoxin contamination values below the limit of detection (LOD) were substituted with LOD/2.^b Sum of aflatoxins includes AFB₁, AFB₂, AFG₁ and AFG₂.^c Sum of fumonisins includes FB₁, FB₂, FB₃ and FB₄.

Median exposures to ochratoxin A were quite similar for the IYC regardless of food item consumed, while family cereal and *Tom bran* contributed to higher exposures to citrinin in the IYC with median values of 0.6 µg/kg bw per day and 0.4 µg/kg bw per day, respectively (Table 3). For exposures to moniliformin and beauvericin, higher median values were estimated from consumption of *Tom bran* as 2 µg/kg bw per day and 0.04 µg/kg bw per day, respectively, than from other complementary food items. It is obvious that the grain combinations (especially maize and peanut which are highly prone to a variety of major mycotoxins, IARC (2015)) used for *Tom bran* formulation at household level contributed to higher mycotoxin levels while the frequencies and quantities of food consumed increased the exposure levels of the IYC to this food. Efforts at household level should be targeted at sourcing alternative grains, adopting good grain handling practices (e.g. drying to safe moisture levels and storage of grains in air-tight metal silos) and revising the proportion of individual grain inputs into *Tom bran* formulation. The source and handling of maize purchased by industries for use in food production, especially foods consumed by IYC, should be strictly monitored to ensure that emerging mycotoxins such as citrinin, which was not previously reported in high quantities in maize in the past decades, do not constitute additional threat to consumers.

The risks of adverse health effects of mycotoxin exposure were estimated for the 110 IYC based on their consumption of the contaminated foods as shown in Fig. 1. In order to categorize the risks from exposures of the IYC to genotoxic and carcinogenic aflatoxin (and the sum of aflatoxins), MOE of 10,000 was applied as a dividing limit. In a similar manner, a MOE of 100 for beauvericin, citrinin and moniliformin was applied as well as the established HBGVs for the sum of fumonisins (group tolerable daily intake (TDI) of 2 µg/kg bw per day) and ochratoxin A (tolerable weekly intake (TWI) of 0.1 µg/kg bw per week). The exposure values that were above the HBGVs or that resulted in lower MOEs were considered as risk (Fig. 1). In the case that exposure values resulted in MOEs higher than the dividing limit of 10,000 for aflatoxins, a low risk was identified. On the other hand, exposures below HBGVs for fumonisins and ochratoxin A or that resulted in MOEs above the dividing limit of 100 in the case of the other mycotoxins were considered no risk. When the exposure estimates were calculated by replacing the analytical results < LOD with LOD/2, 99–100% of all the IYC were at health risk due to the exposures to any of the mycotoxins considered in this study (data not shown). Because this assumption, i.e. all samples < LOD were contaminated with mycotoxins at the level of

LOD/2, was regarded as over-conservative due to the high LODs of the analytical method, the mycotoxin contamination values < LOD were also substituted with zero to obtain another exposure distribution. This latter distribution of exposure values was considered to be more realistic although it may represent an under-estimation of the exposures and consequently the risk (Fig. 1). Approximately 60% of the children were at risk of adverse health effects from exposures to each of aflatoxins and fumonisins while 4, 6, 19 and 25% of the children were at risk from exposures to beauvericin, ochratoxin A, citrinin and moniliformin, respectively. Similar high exposures to aflatoxins and fumonisins were previously reported, although not categorized as done in this study, in children fed complementary foods in Tanzania (Kamala et al., 2017; Kimanya et al., 2010, 2009; Kimanya et al., 2014). The fact that more than one half of the IYC are exposed, and consequently at risk, to fumonisins is noteworthy considering that this toxin has been reported to be linked to neural tube defects (Missmer et al., 2006; Missmer, Hendricks, Suarez, Larsen, & Rothman, 2000) and found to play a role in the impairment of growth in children (Chen et al., 2018; Kimanya et al., 2010; Shirima et al., 2015). Chronic exposure into adulthood may also place these children risk of oesophageal cancer which has been found in many regions where there is chronic exposure to fumonisins (Rheeder et al., 1992; Yoshizawa, Tamashita, & Luo, 1994). The recorded risk levels of the other mycotoxins, especially citrinin and moniliformin, should not be overlooked. Citrinin exposure was, however, not unexpected considering that recently there have been reports of contamination of maize and its products fed to IYC in Nigeria (Okeke et al., 2015; Okeke et al., 2018; Ogara et al., 2017). Overall, it is obvious that high mycotoxin exposures as recorded in this study lead to a risk; this is further substantiated by the similar percentages of the IYC population being highly exposed and at risk of the individual mycotoxins.

With respect to mycotoxin co-exposures from consumption of different complementary food items, patterns are illustrated in Fig. 2. More than 75% of the IYC were co-exposed to at least two mycotoxins and up to four mycotoxins through *Tom bran* consumption while 13% of the IYC were exposed to mycotoxin (one mycotoxin) through consumption of infant formula. In addition, 39% of the IYC were exposed to more than one mycotoxin through *ogi* consumption while mycotoxin co-exposure through family cereal consumption occurred in 69% of the IYC. When the overall consumption of food items was considered, exposure to at least one mycotoxin was found in 75% of the IYC while co-exposures (2–4 mycotoxins) occurred in only 47% of the children, with eight different co-exposure combinations recorded. The commonest exposure and co-exposure patterns recorded for the IYC were FB, AF/FB/CIT, FB/CIT and AF/FB in 19, 18, 10 and 9% of the IYC, respectively. Mycotoxin co-exposures involving more than aflatoxins and fumonisins have been previously suggested from the consumption of

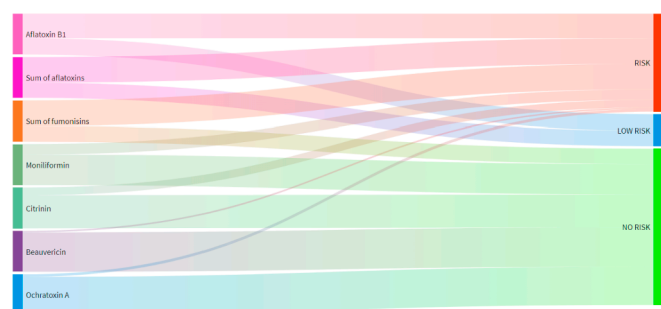


Fig. 1. Mycotoxin risk categorization in 110 infants and young children fed complementary foods in Nigeria. Percentage of IYC population at risk of the adverse effects from dietary mycotoxin exposures: aflatoxin B₁ (60%); sum of aflatoxins (63%); sum of fumonisins (60%); moniliformin (25%); citrinin (19%); beauvericin (4%); ochratoxin A (6%). Risk estimations were based on exposure values where mycotoxin contamination data below the limit of detection (LOD) were substituted with zero. Sum of aflatoxins includes AFB₁, AFB₂, AFG₁ and AFG₂ while sum of fumonisins includes FB₁, FB₂, FB₃ and FB₄. Risk categorization was based on margin of exposure reference points (aflatoxin: 10,000; citrinin: 100; beauvericin: 100; moniliformin: 100) and health based guidance values (fumonisins tolerable daily intake (TDI): 2 µg/kg bw per day; ochratoxin tolerable weekly intake (TWI) 0.1 µg/kg bw per week).

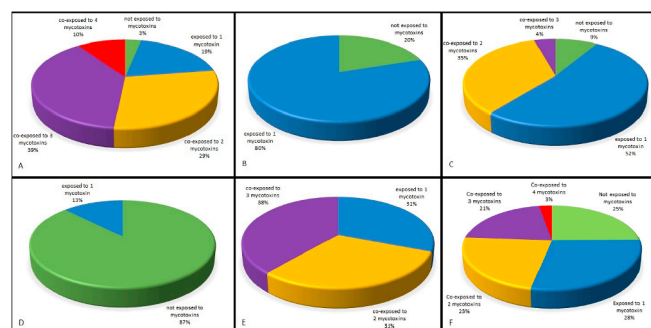


Fig. 2. Co-exposures to mycotoxins in 110 infants and young children in Nigeria based on complementary food preferences. A: *Tom bran*; B: peanut butter; C: *ogi*; D: infant formula; E: family cereal; F: all foods. There was no exposure from milk consumption. Mycotoxin contamination values below the limit of detection (LOD) were substituted with LOD/2.

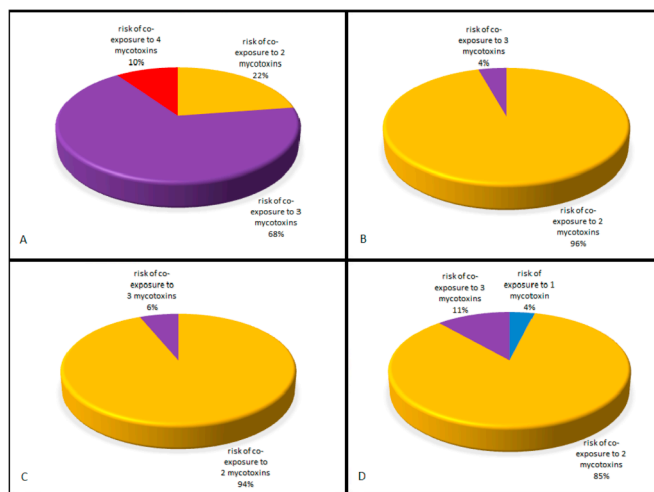


Fig. 3. Risk of co-exposures to mycotoxins in 110 infants and young children in Nigeria based on complementary food preferences. A: *Tom bran*; B: *ogi*; C: infant formula; D: family cereal. Risk of co-exposure to two mycotoxins from peanut butter consumption was 100%. There was no risk of co-exposure from milk consumption. Risk estimations were based on exposure values where mycotoxin contamination data below the limit of detection (LOD) were substituted with LOD/2.

diverse grains, nuts, and their products in SSA (Abia et al., 2013; Adetunji et al., 2014; Ezekiel, Sulyok, Warth, Odebo, & Krska, 2012; Kayode et al., 2013; Ogara et al., 2017; Warth et al., 2012) but only recently suggested in complementary foods in our previous paper (Ojuri et al., 2018). The percentages of IYC co-exposed to mycotoxin mixtures as reported in this study were similar to the reports from cereal-based baby foods sold in Portuguese markets (Alvito et al., 2010), but lower than the 92% co-exposure each reported from commercial infant formula and baby foods from Italian markets (Juan et al., 2014) and breakfast cereals from Portugal (Martins et al., 2018). With respect to patterns of co-exposure, this study presents a unique combination of aflatoxins, citrinin, fumonisins and ochratoxin A that was not reported by the aforementioned publications including the recent paper of Ul Hassan et al. (2018).

In order to determine the risks from co-exposures of the IYC to several mycotoxins (Fig. 3), risk data as described above for single mycotoxin exposures were clustered per individual and evaluated. *Tom bran* consumption resulted in risk of co-exposures of 2–4 mycotoxins with 68% of the IYC being at risk of three co-occurring mycotoxins in this food item. For family cereal, infant formula and *ogi*, 85, 94 and 96% of the IYC were at risk of co-exposure to two mycotoxins, while the lesser populations were co-exposed to three mycotoxins. There was no exposure and risk of co-exposure to these mycotoxins from milk consumption. The risks of co-exposure patterns observed in the IYC were very similar to the patterns described above for co-exposures; thus, indicating the role of food intake in exposure and risk assessment studies. Consequently, it may be necessary to substitute highly prone grains with less prone grains to lower the mycotoxin intake at same time retaining the overall food intake. Fig. 4 highlights the overall risk patterns for mycotoxin co-exposures in the IYC when mycotoxin contamination data below LOD were replaced with LOD/2 and zero. For LOD/2 replacements, 99% of the IYC were at risk of co-exposure from 2 to 4 mycotoxins while only 33% of the children were at risk of same number of mycotoxins when zero was applied as substitute for less than LOD values in food. Generally, the children were mainly at risk of aflatoxins and citrinin with the LOD/2 approach while it was aflatoxins and moniliformin for the LOD = 0 approach. Each of the reported mycotoxins in this study plays significant adverse roles in human toxicology at certain exposure levels but their combined adverse effects in humans, and related health risks have not been established. However,

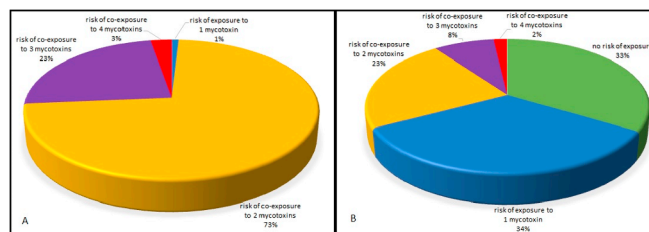


Fig. 4. Risk of mycotoxin co-exposures in 110 infants and young children fed with complementary foods in Nigeria. A: Risk estimations were based on exposure values where mycotoxin contamination data below the limit of detection (LOD) were substituted with LOD/2; B: Risk estimations were based on exposure values where mycotoxin contamination data below the limit of detection (LOD) were substituted with zero.

there are scientific indications that combined adverse effects occur following the dietary exposure to multiple mycotoxins from different toxin classes. For example, mixtures of ochratoxin A and aflatoxins or fumonisins could be detrimental to the human liver cancer (HepG2) cells or PK15 cells, respectively (Golli-Bennour et al., 2010; Klaric et al., 2008), while adding citrinin to the mixture may pose the risk of cytotoxicity of human peripheral blood mononuclei (Stoev et al., 2009) or cause chronic renal disease (Klaric, Rasic, & Peraica, 2013). Since these toxic chemical compounds were found in different mixtures in the various complementary foods, especially the cereal-based foods such as *Tom bran*, family cereal, *ogi* and infant formula, it is necessary to pinpoint the health risks resulting from simultaneous exposures to the different mycotoxin classes. However, till date, the methodology to assess health risks for the combined adverse effects of chemical substances from different classes is yet to be established by risk assessors, such as EFSA and JECFA. Nonetheless, since our study demonstrates that the individual infant is faced with health risks from co-exposure to different mycotoxins on the daily basis, co-exposure should be reflected in the risk assessment process and further in the legislation to avoid negative health effects in this highly vulnerable population, as also concluded by Clarke et al. (2015) and De Ruyck, De Boevre, Huybrechts, and De Saeger (2015).

Considering the high rate of metabolism, lower detoxification capacity and vulnerability of IYC to mycotoxins (Gong et al., 2016; Kostelanska, Sosnovcova, Lacina, & Hajslova, 2010; Weaver, Buckley, & Groopman, 1998), the reported exposure and risk levels from consumption of all food samples in this study are alarming to child health, more especially for those who depend on maize-based complementary foods (*Tom bran*, family cereal and *ogi*). Overall, it can be stated that based on the risk assessment conducted according to the internationally accepted guidelines, there is a significant public health concern associated with high dietary exposures to the mycotoxins among the IYC in this study. Furthermore, based on the co-exposure levels and patterns, the IYC may be at greater risk considering the possible adverse health effects that mixtures of mycotoxins from different classes may induce. A dimension to consider for future studies may include the patterns in combined exposure modeled for the complete set of daily meals for IYC; this is necessary to determine whether a combination of all separate food items composing the full daily diet would result in a higher exposure that may increase the severity of health effects in the IYC consumers, or a lower exposure compared to exposures from individual food items.

3.3.2. Age group-dependent variations in exposure of IYC to mycotoxins

The exposure variations by age group clusters of the 110 IYC are shown in Fig. 5. Except for fumonisins and moniliformin where mean exposures were higher in the children aged less than 12 months, mean exposures to all other mycotoxins were higher in the children within the ages of 12 and 24 months. Mean exposures between the two age groups were significant for only aflatoxins: AFB₁ (2985 ng/kg bw per

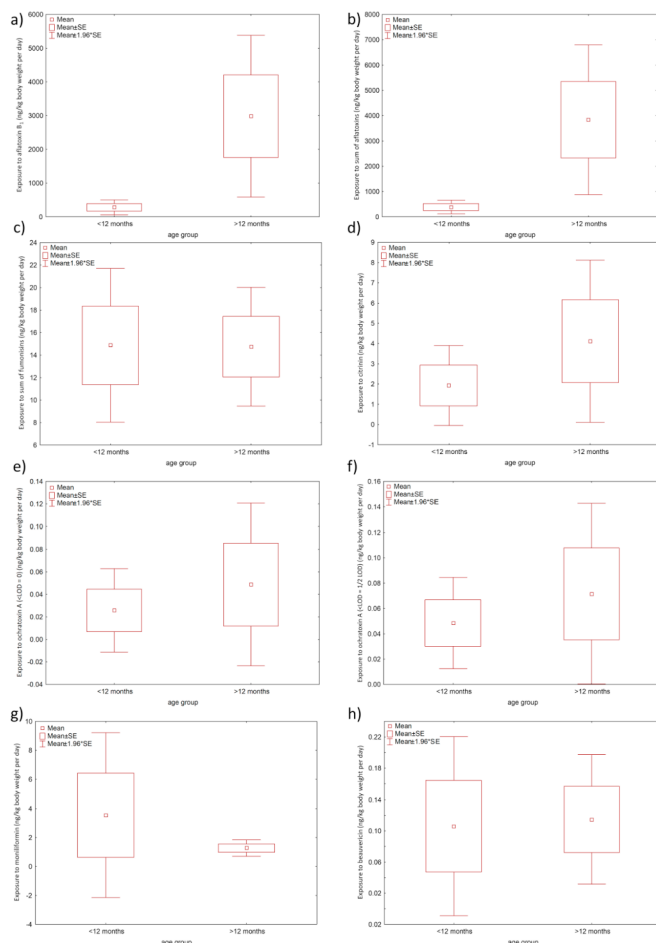


Fig. 5. Age group-dependent boxplot variation in exposure of 110 infants and young children to mycotoxins due to the consumption of complementary foods in Nigeria. Mycotoxin contamination values below the limit of detection (LOD) were substituted with LOD/2.

day for 12–24 months age group vs 282 ng/kg bw per day for < 12 months age group; $p = 0.032$) and sum of aflatoxins (3840 ng/kg bw per day for 12–24 months age group vs 387 ng/kg bw per day for < 12 months age group; $p = 0.027$). This observation is in agreement with the fact that with increased age in children, exposure increases due to the gradual exclusion of breast milk in their diet and introduction of complementary foods made from grains that are highly prone to mycotoxins (Gong et al., 2016; IARC, 2015).

3.4. Influence of awareness and food processing practices on mycotoxin levels in complementary foods

3.4.1. Respondents' awareness of mycotoxin contamination of food

Table 4 presents descriptive data on awareness of mycotoxin contamination of food as obtained from the respondents (caregivers of the IYC) while the result of the logit regression for factors that determine respondents' awareness of mycotoxin contamination of food are shown in Table 5. Only 33% of the respondents were aware of mycotoxin contamination of foods; each respondent indicated several sources of awareness. Seminars, internet sources, interactions with family/friends, and ante-/post-natal visits to clinic constituted the major sources of awareness (Table 4). The educational level of the respondents had no significant influence on their awareness of mycotoxin contamination of food (Table 5); this can mainly be as a result of insufficient public information about mycotoxins contamination of food. On the other hand, the respondents' perceived risk of mycotoxin contamination from

Table 4

Basic descriptive statistics of respondents' awareness to mycotoxins.

Variables	Frequency (n = 110)	%
<i>Possible awareness of mycotoxins (dummy)</i>		
1 = Yes	36 ^a	32.7
0 = No	74 ^a	67.3
<i>Source of awareness (N = 36^a; n = 66^b)</i>		
Clinic (ante-/post -natal)	15	22.7
Mass media	4	6.1
Internet	16	24.2
Relatives/Friends	14	21.2
Seminar	17	25.8

^a Number of respondents who were aware (Yes) or not aware (No) of mycotoxins.

^b Number of overall responses due to multiple sources of awareness indicated by some of the respondents who were aware.

Table 5

Logit regression output for factors influencing respondents' awareness of mycotoxin contamination of complementary foods.

Variables	β -Coefficient	Std. Error
Use of food product label (dummy: Use = 1; otherwise = 0)	0.12	0.46
Experience with contaminated food (dummy: yes = 1; otherwise = 0)	20.24*	9.3
Respondent's education (years)	4.70	120.6
Constant	−95.65	21430.8

Chi-square = 30.266*; $R^2 = 0.335$; $-2 \text{ Log likelihood} = 108.82$.

previous personal experience with contaminated food had significant ($p < 0.05$) positive influence on awareness (Table 5). The low level of awareness/minimal knowledge of food safety and mycotoxin issues prevalent amongst caregivers of the IYC regardless of their educational level as depicted by their ignorance of food product labels amongst other feeding practices (data not shown) agrees with reports of low mycotoxin awareness in Nigeria reported in previous studies (Adekoya et al., 2017; Ezekiel et al., 2013). Respondents with previous experience of contaminated food are likely to be more deliberate in accessing information on food safety. This may be responsible for the positive relationship between respondents' perceived risk of mycotoxin contamination from personal experience and awareness as reported.

3.4.2. Regression analysis of respondents' awareness and selected food processing practices and mycotoxin levels in the household-formulated complementary foods

The regression outputs of the respondents' awareness and selected food processing practices with the levels of sum of aflatoxins, citrinin and sum of fumonisins found in the household-formulated complementary food samples are presented in Table 6. The selected food processing practices included length of food storage, type of home-made food processed and fed to the IYC, food storage material (data not shown), and ability of respondents to identify mouldy food. The length of food storage was found to significantly ($p = 0.02$) influence the levels of only the sum of aflatoxins quantified in the household-formulated complementary foods; this confirms that poor storage of grains is a critical factor to aflatoxin accumulation in cereals (Adetunji et al., 2014). Critical examination of the beta coefficients of the two household-formulated foods (*Tom bran* and *ogi*) suggests that IYC who were fed with *Tom bran* were at higher risk (β -coefficient: 51.4; $p = 0.01$) of aflatoxins exposure than IYC fed with *ogi*, while those fed with *ogi* were at higher risk (β -coefficient: -193.4 ; $p = 0.04$) of exposure to fumonisins than IYC fed with *Tom bran*; this further confirms the food contamination data for both mycotoxins as well as exposure patterns described in the previous sections of this paper. It is imperative to mention at this point that the aflatoxin issue in *Tom bran* can be minimized

Table 6

Regression output for influence of food processing practices on aflatoxin, citrinin and fumonisin levels in the complementary foods.

Mycotoxin	Variable	β -coefficient	t-value	Significance
Sum of aflatoxins ^a	Length of food storage	20.62 [*]	2.80	0.02
	Type of home-made food fed to IYC (1 = <i>Tom bran</i> , 0 = <i>Ogi</i>)	51.43 [*]	3.30	0.01
	Mycotoxin awareness (1 = Yes, 0 = No)	−0.47	−0.01	0.99
	Ability to identify mouldy food (1 = Yes, 0 = No)	−35.50	−0.81	0.42
Citrinin ^b	Length of food storage	3.88	0.19	0.85
	Type of home-made food fed to IYC (1 = <i>Tom bran</i> , 0 = <i>Ogi</i>)	89.02	1.27	0.21
	Mycotoxin awareness (1 = Yes, 0 = No)	88.85	1.06	0.30
	Ability to identify mouldy food (1 = Yes, 0 = No)	−2.35	−0.03	0.98
Sum of fumonisins ^c	Length of food storage	−17.80	−0.67	0.51
	Type of home-made food fed to IYC (1 = <i>Tom bran</i> , 0 = <i>Ogi</i>)	−193.39 [*]	−2.10	0.04
	Mycotoxin awareness (1 = Yes, 0 = No)	−282.33 [*]	−2.55	0.02
	Ability to identify mouldy food (1 = Yes, 0 = No)	−233.17 [*]	−2.28	0.03

^a Durbin Watson = 1.99; Adjusted R² = 0.61.^b Durbin Watson = 2.33; Adjusted R² = 0.63.^c Durbin Watson = 2.26; Adjusted R² = 0.40.* Significance at $p \leq 0.05$.

drastically by finding cheap and nutritious alternatives to maize and peanut which are the chief susceptible grains, whilst for *ogi* maize needs replacement (please check Ojuri et al., 2018 for suggested small grain substitutes). None of the independent variables significantly influenced citrinin levels in the food samples. Further result suggests that the respondents' awareness of mycotoxins ($p = 0.02$) and their ability to identify mouldy food ($p = 0.03$) significantly influenced a reduction in the level of fumonisins in the food sample. Considering that the proportion of households in this study that fed their IYC with home-made complementary food alone or in combination with industrially-processed foods were high, deliberate steps are required to create awareness on the effect of adopting good food processing practices at the household level.

4. Conclusion and risk management advice

This is the first study to report a comparison of mycotoxins in complementary foods processed at household and industrial levels, and assessment of co-exposures and risks of co-exposures in IYC consuming these diets in Nigeria. Furthermore, we elucidated the influence of awareness and processing practices on toxin levels in the foods. Household-formulated complementary foods contained higher levels of several mycotoxins, excluding fumonisins, compared to industrially-processed foods. Exposure estimates from consumption of the individual complementary food items were high, with the foods containing maize being the most culprits. In addition, high proportions of the IYC were co-exposed to eight different mycotoxin combinations. The proportion of caregivers of the IYC who were aware of mycotoxin issues was low, and food processing practices, particularly at household level, negatively increased mycotoxin levels in the complementary food samples. In view of the findings of this study, a set of integrated approaches is recommended for inclusion in the risk management plan for minimizing mycotoxin contamination in the food chain for IYC in Nigeria. Some suggestions include:

- encourage crop/grain farmers on good agricultural practices (e.g. sourcing high quality seeds for planting; timely sowing, weeding and harvesting of crops; use of appropriate pesticides including biopesticides) that will keep mycotoxin contamination in the field at the barest minimum;
- adopt good crop postharvest handling and processing practices (drying of grains to safe moisture levels, drying in proper environment (e.g. on clean slabs protected from the bare ground or using mechanical dryers), timely transportation of crops under good conditions, proper grain storage in air-tight metal silos, sorting/cleaning of grains);

- dietary diversity and grain replacement/substitution are required for household-formulated complementary foods, especially when mixed grains are involved;
- strict surveillance and monitoring of industrially-processed foods, especially those intended for IYC, should be prioritized by the regulatory agencies in the country. Mycotoxin regulations for complementary foods in Nigeria require revision to include other mycotoxins, at least those regulated by the EU; this will give a boost to surveillance activities and keep food processors more cautious about their responsibilities to protect consumer health;
- routine food safety and mycotoxin awareness/educational interventions programs are recommended for mothers, care-givers of IYC, crop growers/farmers, and food processors and handlers. Deliberate efforts at incorporating food safety topics (including mycotoxins) in educational curricula beginning at the secondary school level should be prioritized.

Conflicts of interest

The authors declare they have no competing financial interests.

Acknowledgements

This study was supported with funding from the European Union's Horizon 2020 research and innovation programme (grant agreement No.: 692195 (MultiCoop)). The households that supplied food items are greatly appreciated.

References

- Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., et al. (2013). Determination of multi-mycotoxin occurrence in cereals, nuts, and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control*, 31, 438–453.
- Adekoya, I., Njobeh, P., Obadina, A., Chilaka, C., Okoth, S., De Boevre, M., et al. (2017). Awareness and prevalence of mycotoxin contamination in selected Nigerian fermented foods. *Toxins*, 9(11), 363.
- Adetunji, M. C., Atanda, O. O., Ezekiel, C. N., Sulyok, M., Warth, B., Beltran, E., et al. (2014). Fungal and bacterial metabolites of stored maize (*Zea mays*, L.) from five agro-ecological zones of Nigeria. *Mycotoxin Research*, 30, 89–102.
- Alvito, P. C., Sizoo, E. A., Almeida, C. M. M., & Egmond, H. P. V. (2010). Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal. *Food Analytical Methods*, 3, 22–30.
- Babalola, D. A., Babalola, Y. T., & Bassey, M. E. (2010). Determinants of consumers' preference for information sources for food safety: Evidence from Akwa Ibom state. *International Journal of Information Resources and Knowledge Management*, 1(1), 33–41.
- Baydar, T., Erkekoglu, P., Sipahi, H., & Sahin, G. (2007). Aflatoxin B₁, M₁ and ochratoxin A levels in infant formulae and baby foods marketed in Ankara, Turkey. *Journal of Food and Drug Analysis*, 15(1), 89–92.
- Benford, D., Bolger, P. M., Carthew, P., Coulet, M., DiNovi, M., & Leblanc, J. (2010). Application of the margin of exposure (MOE) approach to substances in food that are

- genotoxic and carcinogenic. *Food and Chemical Toxicology*, 8, S2–S24.
- Chen, C., Mitchell, N. J., Gratz, J., Houpt, E. R., Gong, Y., Egner, P. A., et al. (2018). Exposure to aflatoxin and fumonisin in children at risk for growth impairment in rural Tanzania. *Environment International*, 115, 29–37.
- Clarke, R., Connolly, L., Frizzell, C., & Elliott, C. T. (2014). Cytotoxic assessment of the regulated, co-existing mycotoxins aflatoxin B₁, fumonisin B₁ and ochratoxin, in single, binary and tertiary mixtures. *Toxicology*, 90, 70–81.
- Clarke, R., Connolly, L., Frizzell, C., & Elliott, C. T. (2015). High content analysis: A sensitive tool to detect and quantify cytotoxic, synergistic and antagonistic effects of chemical contaminants in foods. *Toxicology Letters*, 233(3), 278–286.
- Codex Alimentarius (1989). Guidelines for the simple evaluation of dietary exposure to food additives CAC/GL 3-1989. Adopted 1989. Revision 2014. (formerly guidelines for the simple evaluation of food additive intake). Codex Alimentarius international food standards. Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO). Available online: <http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/>.
- Creppy, E. E., Chirappa, P., Baudrimont, I., Borraci, P., Moukha, S., & Carratu, M. R. (2004). Synergistic effects of fumonisin B₁ and ochratoxin A: Are *in vitro* cytotoxicity data predictive of *in vivo* acute toxicity? *Toxicology*, 201, 115–123.
- De Ruyck, K., De Boever, M., Huybrechts, I., & De Saeger, S. (2015). Dietary mycotoxins, co-exposure, and carcinogenesis in humans: Short review. *Mutation Research: Reviews in Mutation Research*, 766, 32–41.
- EFSA (European Food Safety Authority) (2005). Opinion of the scientific committee on a request from EFSA related to A harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA Journal*, 282, 1–31.
- EFSA (European Food Safety Authority) (2007). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazel nuts and pistachios and derived products. *EFSA Journal*, 446, 1–127.
- EFSA (European Food Safety Authority) (2012). Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. *EFSA Journal*, 10(3), 2605.
- Ezekiel, C. N., Sulyok, M., Babalola, D. A., Warth, B., Ezekiel, V. C., & Krska, R. (2013). Incidence and consumer awareness of toxigenic *Aspergillus* section *Flavi* and aflatoxin B₁ in peanut cake from Nigeria. *Food Control*, 30(2), 596–601.
- Ezekiel, C. N., Sulyok, M., Warth, B., Odebo, A. C., & Krska, R. (2012). Natural occurrence of mycotoxins in peanut cake from Nigeria. *Food Control*, 27, 338–342.
- Golli-Bennour, E. E., Kouidhi, B., Bouslimi, A., Abid-Essefi, S., Hassen, W., & Bacha, H. (2010). Cytotoxicity and genotoxicity induced by aflatoxin B₁, ochratoxin A, and their combination in cultured Vero cells. *Journal of Biochemistry and Molecular Toxicology*, 24, 42–50.
- Gong, Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P. C., & Hall, A. J. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: Cross sectional study. *British Medical Journal*, 325, 20–21.
- Gong, Y., Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. C., & Hall, A. J. (2004). Post-weaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, west Africa. *Environmental Health Perspectives*, 112, 1334–1338.
- Gong, Y. Y., Watson, S., & Routledge, M. N. (2016). Aflatoxin exposure and associated human health effects, a review of epidemiological studies. *Food Safety*, 4(1), 14–27.
- Gujarati, D. N. (2003). *Essentials of econometrics* (3rd ed.). Singapore: McGraw-Hill.
- IARC (International Agency for Research on Cancer) (2015). Mycotoxin control in low and middle income countries. In C. P. Wild, J. D. Miller, & J. D. Groopman (Eds.). *IARC Working Group Report No. 9*, Lyon.
- IPCS (The International Programme on Chemical Safety) (2009). Principles and methods for the risk assessment of chemicals in food. A joint publication of the food and agriculture organization of the united Nations and the world health organization. *Environmental Health Criteria* 240. <http://www.who.int/foodsafety/publications/chemical-food/en/>.
- Juan, C., Raiola, A., Mänes, J., & Ritieni, A. (2014). Presence of mycotoxin in commercial infant formulas and baby foods from Italian market. *Food Control*, 39, 227–236.
- Kabak, B. (2009). Ochratoxin A in cereal-derived products in Turkey: Occurrence and exposure assessment. *Food and Chemical Toxicology*, 47, 348–352.
- Kamala, A., Kimanya, M., Lachat, C., Jacxsens, L., Haesaert, G., Kolsteren, P., et al. (2017). Risk of exposure to multiple mycotoxins from maize-based complementary foods in Tanzania. *Journal of Agricultural and Food Chemistry*, 65(33), 7106–7114.
- Kang'ethe et al., 2017 Kang'ethe, E. K., Korhonen, H., Marimba, K. A., Nduhiu, G., Mungatu, J. K., Okoth, S. A., et al. (2017). Management and mitigation of health risks associated with the occurrence of mycotoxins along the maize value chain in two counties in Kenya. *Food Quality and Safety*, 1(4), 268–274.
- Kayode, O. F., Sulyok, M., Papohunda, S. O., Ezekiel, C. N., Krska, R., & Oguntola, C. R. B. (2013). Mycotoxins and fungal metabolites in groundnut- and maize-based snacks from Nigeria. *Food Additives and Contaminants: Part B*, 6, 294–300.
- Kimanya, M. E., De Meulenaer, B., Baert, K., Tiisekwa, B., Camp, J. V., Samapundo, S., et al. (2009). Exposure of infants to fumonisins in maize-based complementary foods in rural Tanzania. *Molecular Nutrition & Food Research*, 53, 667–674.
- Kimanya, M. E., De Meulenaer, B., Baert, K., Tiisekwa, B., Camp, J. V., Samapundo, S., et al. (2010). Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition & Food Research*, 54, 1659–1667.
- Kimanya, M., De Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., & Kolsteren, P. (2008). Human exposure to fumonisins from home grown maize in Tanzania. *World Mycotoxin Journal*, 1, 307–313.
- Kimanya, M. E., Shirima, C. P., Magoha, H., Shewiyo, D. H., Meulenaer, B. D., Kolsteren, P., et al. (2014). Co-exposures of aflatoxins with deoxynivalenol and fumonisins from maize based complementary foods in Rombo, Northern Tanzania. *Food Control*, 41, 76–81.
- Klarić, M. S., Rasic, D., & Peraica, M. (2013). Deleterious effects of mycotoxin combinations involving ochratoxin A. *Toxins*, 5, 1965–1987.
- Klarić, M. S., Rumora, L., Ljubanović, D., & Pepeljnjak, S. (2008). Cytotoxicity and apoptosis induced by fumonisin B₁, beauvericin and ochratoxin A in porcine kidney PK15 cells: Effects of individual and combined treatment. *Archives of Toxicology*, 82, 247–255.
- Klarić, M. S., Zeljezic, D., Rumora, L., Peraica, M., Pepeljnjak, S., & Domijan, A. M. (2012). A potential role of calcium in apoptosis and aberrant chromatin forms in porcine kidney PK15 cells induced by individual and combined ochratoxin A and citrinin. *Archives of Toxicology*, 86, 97–107.
- Kostelanska, M., Sosnovcova, I., Lacina, O., & Hajslova, J. (2010). Determination of mycotoxins in infant and baby food using UPLC-MS/MS analytical method. *Prague*, 6, 1–5.
- Lindsay, A. C., Machado, M. T., Sussner, K. M., Hardwick, C. K., & Peterson, K. E. (2008). Infant-feeding practices and beliefs about complementary feeding among low-income Brazilian mothers: A qualitative study. *Food and Nutrition Bulletin*, 29(1), 15–24.
- Malachová, A., Sulyok, M., Beltrán, E., Berthiller, F., & Krska, R. (2014). Optimization and validation of a quantitative liquid chromatography–tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. *Journal of Chromatography A*, 1362, 145–156.
- Martani, J. L. (2014). Mycotoxin exposure and infant and young child growth in Africa: What do we know? *Annals of Nutrition & Metabolism*, 64(2), 42–52.
- Martins, A. C., Assuncao, R., Cunha, S. C., Fernandes, J. O., Jager, A., Petta, T., et al. (2018). Assessment of multiple mycotoxins in breakfast cereals available in the Portuguese market. *Food Chemistry*, 239, 132–140.
- Matumba, L., Monjerezi, M., Biswick, T., Mwatseteza, J., Makumba, W., Kamangira, D., et al. (2014). A survey of the incidence and level of aflatoxin contamination in a range of locally and imported processed foods on Malawian retail market. *Food Control*, 39, 87–91.
- Missmer, S., Hendricks, K. A., Suarez, L., Larsen, R. D., & Rothman, K. J. (2000). Fumonisin and neural tube defects: Preliminary results from the Texas department of health. *Epidemiology*, 11, 183–184.
- Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H., Jr., Rothman, K. J., et al. (2006). Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. *Environmental Health Perspectives*, 114, 237–241.
- Mngqawa, P., Shephard, G. S., Green, I. R., Ngobeni, S. H., de Rijk, T. C., & Katerere, D. R. (2016). Mycotoxin contamination of home-grown maize in rural northern South Africa (Limpopo and Mpumalanga Provinces). *Food Additives and Contaminants: Part B*, 9(1), 38–45.
- Njumbe-Eidage, E., Hell, K., & De Saeger, S. (2014). A comprehensive study to explore differences in mycotoxin pattern from agro-ecological regions through maize, peanut and cassava products: A case study, Cameroon. *Journal of Agricultural and Food Chemistry*, 62, 4789–4797.
- Ogara, I. M., Zarafi, A. B., Alabi, O., Banwo, O., Ezekiel, C. N., Warth, B., et al. (2017). Mycotoxin patterns in ear rot-infected maize: A comprehensive case study in Nigeria. *Food Control*, 73, 1159–1168.
- Ojuri, O. T., Ezekiel, C. N., Sulyok, M., Ezeokoli, O. T., Oyedele, O. A., Ayeni, K. I., et al. (2018). Assessing the mycotoxicological risk from consumption of complementary foods by infants and young children in Nigeria. *Food and Chemical Toxicology*, 121, 37–50.
- Okeke, C. A., Ezekiel, C. N., Nwangburuka, C. C., Sulyok, M., Ezeamagu, C. O., Adeleke, R. A., et al. (2015). Bacterial diversity and mycotoxin reduction during maize fermentation (steeping) for *ogi* production. *Frontiers in Microbiology*, 6, 1402.
- Okeke, C. A., Ezekiel, C. N., Sulyok, M., Ogunremi, O. R., Ezeamagu, C. O., Sarkanj, B., et al. (2018). Traditional processing impacts mycotoxin levels and nutritional value of *ogi* – a maize-based complementary food. *Food Control*, 86, 224–233.
- Okoth, S. A., & Ohingo, M. (2004). Dietary aflatoxin exposure and impaired growth in young children from kisumu district, Kenya: Cross sectional study. *African Journal of Health Sciences*, 11(1–2), 43–54.
- Oyedele, O. A., Ezekiel, C. N., Sulyok, M., Adetunji, M. C., Warth, B., Atanda, O. O., et al. (2017). Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. *International Journal of Food Microbiology*, 251, 24–32.
- Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Shephard, G. S., & Van Schalkwyk, D. J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology*, 82, 353–357.
- Shirima, P. C., Kimanya, E. M., Routledge, M. N., Srey, C., Kinabo, L. J., Humpf, H., et al. (2015). A prospective study of growth and biomarkers of exposure to aflatoxins and fumonisins during childhood in Tanzania. *Environmental Health Perspectives*, 123, 173–178.
- Stoev, S. D., Denev, S., Dutton, M., & Nkosi, B. (2009). Cytotoxic effect of some mycotoxins and their combinations on human peripheral blood mononuclear cells as measured by MTT assay. *The Open Toxicology Journal*, 2, 1–8.
- Sulyok, M., Krska, R., & Schuhmacher, R. (2007). A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Analytical and Bioanalytical Chemistry*, 389, 1505–1523.
- Tam, J., Mankotia, M., Mably, M., Pantazopoulos, P., Neil, R. J., Calway, P., et al. (2006). Survey of breakfast and infant cereals for aflatoxins B₁, B₂, G₁ and G₂. *Food Additives & Contaminants: Part A*, 23(7), 693–699.
- Turner, P. C. (2013). The molecular epidemiology of chronic aflatoxin driven impaired child growth. *Scientific*. <https://doi.org/10.1155/2013/152879>.
- Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y. Y., Hall, A. J., Prentice, A. M., et al. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, 36, 1119–1125.
- Turner, P. C., Moore, S. E., Hall, A. J., Prentice, A. M., & Wild, C. P. (2003). Modification

- of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives*, 111, 217–220.
- Ul Hassan, Z., Al Thani, R., Atia, F. A., Al Meer, S., Migheli, Q., & Jaoua, S. (2018). Co-occurrence of mycotoxins in commercial formula milk and cereal-based baby food on the Qatar market. *Food Additives and Contaminants: Part B*. <https://doi.org/10.1080/19393210.2018.1437785>.
- Warth, B., Parich, A., Atehnkeng, J., Bandyopadhyay, R., Schuhmacher, R., Sulyok, M., et al. (2012). Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method. *Journal of Agricultural and Food Chemistry*, 60, 9352–9363.
- Weaver, V. M., Buckley, T. J., & Groopman, J. D. (1998). Approaches to environmental exposure assessment in children. *Environmental Health Perspectives*, 106, 827–832.
- Yoshizawa, T., Yamashita, A., & Luo, Y. (1994). Fumonisin occurrence in corn from high and low risk areas for human esophageal cancer in China. *Applied and Environmental Microbiology*, 60, 1626–1629.